Development of Protein Loaded Redox-Active Injectable Gel by Polyion Complex for Pharmaceutical Applications

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Development of Protein Loaded Redox-Active Injectable Gel by Polyion Complex for Pharmaceutical Applications

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Abstract

For more than 100 years, innovative drug development has been performed mainly by organic synthetic chemistry. In recent years, drug development has shifted to new areas such as molecular targeting drugs. Cancer immunotherapy is one of the most attractive targets for new drug discovery. Because immunotherapy is based on the stimulation of a patient's own immune system, it is believed to be patient friendly. However, there are several hurdles in developing bioactive proteins for immunotherapy because of the severe systemic toxicities, called "immune-related adverse events", caused by their conventional injection formulations [1-5]. For example, interleukin-12 (IL-12) has the significant antitumor activity, however the administration of IL-12 by the conventional formulations resulted in excessive systemic toxicities including deaths in a clinical trial [1, 6]. The severe adverse effects prevent administration of an effective dose of IL-12 as an antitumor immune therapeutics [7].

Local delivery is an attractive approach to achieve the therapeutic concentration of bioactive proteins in the tumor microenvironment, avoiding an excess of maximum tolerated dose [8-10]. In order to improve controlled protein local delivery, several trials such as those with protein/polymer mixtures have been reported. However, these approaches are not well established due to the limited extension of retention time at the injection site and low mechanical strength [11, 12]. Injectable stimuli-responsive hydrogels are one of the promising candidates for local protein delivery because they are flowable as aqueous solutions before administration. Once the flowable solution is injected subcutaneously, it immediately converts to gel. Hydrophobic-hydrophilic-hydrophobic (ABA-type triblock) copolymers as a matrix for an injectable gel system have been widely studied for the controlled release of drugs [12]. However, it is difficult to encapsulate charged drugs effectively in the gel and provide sustained releases of them by using this type of copolymers because of poor interactions between hydrophobic chains in the copolymers and charged drugs [13-15].

Recently our research group has developed and reported a redox-active injectable gel (RIG) system by using poly[4-(2,2,6,6-tetramethylpiperidine-N-oxyl)aminomethylstyrene]-b-poly-(ethylene glycol)-b-poly[4-(2,2,6,6-tetramethylpiperidine-Noxyl) aminomethylstyrene] (PMNT−PEG−PMNT) triblock

copolymer, which possesses reactive oxygen species (ROS) scavenging nitroxide radicals as side chains on the PMNT segment. The cationic PMNT segment in PMNT−PEG−PMNT forms polyion complexes (PIC) with anionic poly(acrylic acid) (PAAc) to form flower-like micelles, which exhibit temperature- and ionic strength-responsive irreversible gelation under physiological conditions. The RIG showed a suppression of ROS induced adverse events such as an inflammation related to oxidative stress by ROS scavenging molecules in the side chains of PMNT-PEG-PMNT in the RIG [16].

In this study, to solve these issues, the author designed and developed protein-loaded, redox-active, injectable, gel (RIG) formed by a polyion complex (protein@RIG) for an ideal local protein therapy, which is formed by a polyion complex (PIC) comprising three components, viz., cationic polyamine-poly(ethylene glycol)-polyamine triblock copolymer possessing ROS-scavenging moieties as side chains (PMNT-PEG-PMNT); anionic poly(acrylic acid) (PAAc); and a protein (Figure 1). Since the driving force for the formation of the PIC flower micelle is electrostatic interaction, it was expected that the micelle could encapsulate charged compounds, including proteins and peptides, and suppress rapid diffusion of the encapsulated compounds by causing the gel formation *in vivo*. One of the other features of the PIC-flower micelle is the elimination of ROS by the gel itself. Since nitroxide radicals, which served as a side chain of the PMNT segment in the block copolymer by forming covalent bonds, catalytically eliminate ROS, it was anticipated that the redox-active injectable gel system could suppress ROS-induced adverse events [16, 17].

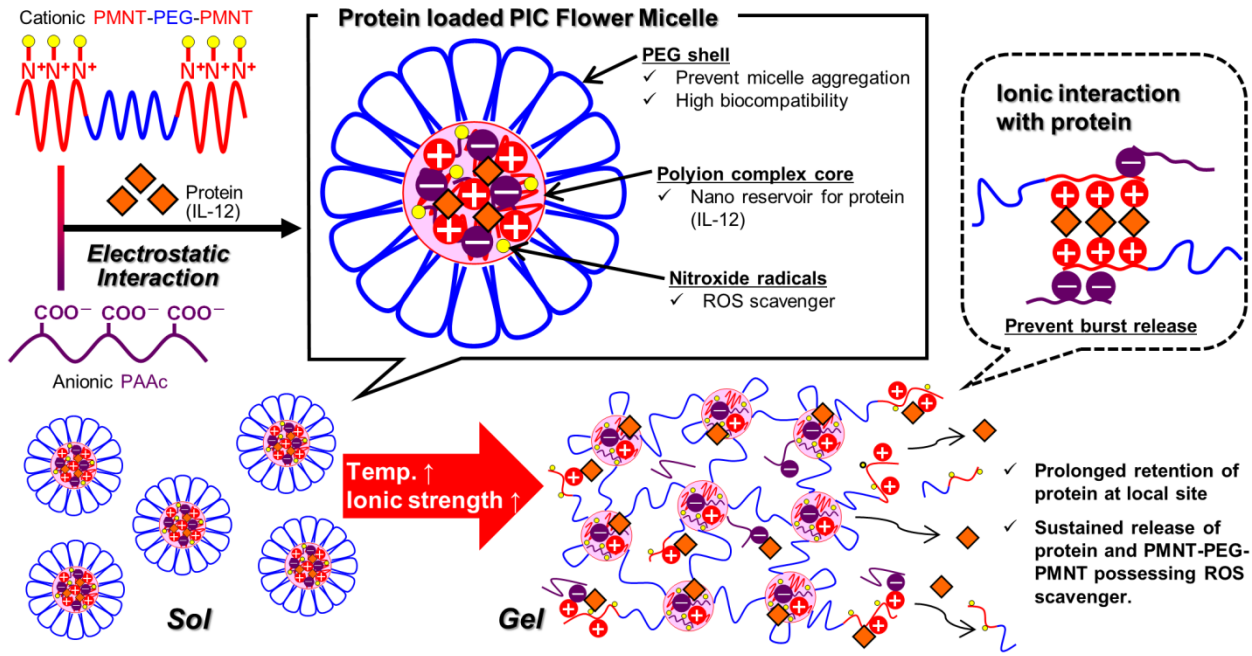

Figure 1. Schematic illustration of protein-loaded redox-active injectable gel (RIG). Polyion complex (PIC) flower micelles are formed by self–assembly via electrostatic interaction between cationic polyamine-poly(ethylene glycol)-polyamine triblock copolymer (PMNT-PEG-PMNT) possessing nitroxide radicals, which eliminate reactive oxygen species (ROS), as side chains of polyamine segments, anionic poly(acrylic acid), and protein. Protein-loaded PIC flower micelle solution shows irreversible sol−gel transition with increasing temperature and ionic strength. RIG can provide prolonged retention of the protein at a local site and a sustained release of the protein without a significant initial burst owing to ionic interactions between the charged polymers and the protein.

The material properties of dual stimuli-responsive redox-active injectable gel (RIG) by polyion complex based flower micelles was investigated in detail. Cationic PMNT-PEG-PMNT and anionic PAAc formed polyion complexes (PIC) flower micelles without aggregation, which was confirmed by dynamic light scattering (DLS) measurement and fluorescence resonance energy transfer (FRET) analysis. The author confirmed that the PIC flower micelles exhibited irreversible sol-gel phase transitions with increasing both temperatures and ionic strengths by rheological evaluation using a rheometer. The PIC flower micelles had a low viscosity at room temperature. The viscosity increased with increasing temperatures and ionic strengths, and the RIG was formed under physiological condition. The RIG was able to provide sustained release of an anionic model drug for more than 4 weeks without an initial burst.

The author designed and developed a long-acting, protein-loaded, redox-active, injectable gel formed by a polyion complex for local protein therapeutics. The author evaluated the material properties of the protein-loaded RIG / PIC flower micelles using various types of proteins with different molecular weight and isoelectronic point. The protein-loaded PIC flower micelles exhibited sol−gel phase transition with increasing temperature and formed the gel under physiological condition. The RIG was able to incorporate various kinds of proteins and provide a sustained release of the protein without a significant initial burst regardless of protein types *in vitro*, and much longer retention of the protein at the local injection site in mice than the naked protein. The IL-12-loaded RIG showed remarkable tumor growth inhibition in tumor-bearing mice, compared to the naked IL-12, when administrated by subcutaneous injection at the adjacent site to the tumor. In addition, IL-12-loaded RIG suppressed lymphopenia and the increase of TNF-α level in the liver of the mice, which were caused by IL-12–induced ROS.

In this thesis, the author revealed that protein-loaded RIG had the potential as a platform technology for an injectable sustained release carrier for proteins to provide high therapeutic effect with suppressing the adverse effects.

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